E. coli
E. coli
Shiga Toxin Methods and Protocols

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Preface

The study of the pathogenesis of Shiga toxin-producing *Escherichia coli* (STEC) infections encompasses many different disciplines, including clinical microbiology, diagnostics, animal ecology, and food safety, as well as the cellular microbiology of both bacterial pathogenesis and the mechanisms of toxin action. *E. coli: Shiga Toxin Methods and Protocols* aims to bring together a number of experts from each of these varied fields in order to outline some of the basic protocols for the diagnosis and study of STEC pathogenesis. We hope that our book will prove a valuable resource for the clinical microbiologist as well as the cellular microbiologist.

For the clinical microbiologist, our aim is to detail a number of current protocols for the detection of STEC in patient samples, each of which have their own advantages. Chapter 1 provides an introduction into the medical significance of STEC infections. Chapters 2–7 follow with protocols for the diagnosis and detection of STEC bacteria in patient and animal samples.

For the cellular microbiologist, we have brought together a number of experts from basic microbiologists to cell biologists to provide different protocols useful in studying the varied aspects of STEC pathogenesis. Chapters 8–13 concentrate on the cellular microbiology of STEC infections, describing protocols to study host–pathogen interactions as well as studies on the hemolysin of STEC. In Chapters 14–22, various protocols are described for studying the details of Shiga toxin (Stx) biology, from the purification of the toxin to studies of the effects of Stx on various host cell functions. Finally Chapters 23–25 provide detailed protocols for the study of STEC-mediated disease in various animal models.

The format of the chapters will be familiar to those who have used other volumes in the Methods in Molecular Medicine series. The Notes section at the end of each chapter pays particular attention to detailing the potential problems that may be encountered, as well as providing alternate methods for the protocols described.

Finally, we hope *E. coli: Shiga Toxin Methods and Protocols* will benefit those interested in both the clinical and pathological aspects of STEC infections, as well as provide a number of valuable protocols for those
researchers studying host–pathogen interactions. We would like to thank the contributing authors as well as John Walker and the staff at Humana Press for their assistance in putting this volume together.

Dana Philpott
Frank Ebel
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The Medical Significance of Shiga Toxin-Producing Escherichia coli Infections

An Overview

Mohamed A. Karmali

1. Introduction

Shiga toxin (Stx)-producing Escherichia coli (STEC), also referred to as Verocytotoxin-producing E. coli (VTEC) (1), are causes of a major, potentially fatal, zoonotic food-borne illness whose clinical spectrum includes non-specific diarrhea, hemorrhagic colitis, and the hemolytic uremic syndrome (HUS) (2–6). The occurrence of massive outbreaks of STEC infection, especially resulting from the most common serotype, O157:H7, and the risk of developing HUS, the leading cause of acute renal failure in children, make STEC infection a public health problem of serious concern (2,5,7). Up to 40% of the patients with HUS develop long-term renal dysfunction and about 3–5% of patients die during the acute phase of the disease (8–11). There is no specific treatment for HUS, and vaccines to prevent the disease are not yet available. The purpose of this overview is to highlight the public health impact, epidemiology, and clinicopathological features of STEC infection.

2. Public Health Impact and Epidemiology of STEC Infection

Shiga toxin-producing E. coli infection is usually acquired by the ingestion of contaminated food or water or by person-to-person transmission (2,5,7). The natural reservoir of STEC is the intestinal tracts of domestic animals, particularly cattle and other ruminants. Sources for human infection include foods of animal origin such as meats (especially ground beef), and unpasteurized...
milk, and other vehicles that have probably been cross-contaminated with STEC, such as fresh-pressed apple cider, yogurt, and vegetables such as lettuce, radish sprouts, alfalfa sprouts, and tomatoes (2,5,7). Person-to-person transmission, facilitated by a low infectious dose, is common. Waterborne transmission and acquisition of infection in the rural setting and via contact with infected animals are becoming increasingly recognized. STEC infection occurs, typically, during the summer and fall and affects mostly young children, although the elderly also have an increased risk of infection (2,5,7).

Although over 200 different OH serotypes of STEC have been associated with human illness (5), the vast majority of reported outbreaks and sporadic cases in humans have been associated with serotype O157:H7 (2,5,7). Other STEC serotypes that have been associated with outbreaks include O26:H11, O103:H2, O104:H21, O111:H2, and O145:H2. Outbreaks with cases of HUS have occurred almost exclusively with serotypes that exhibit the characteristic attaching and effacing (A/E) cytopathology, which is encoded for by the LEE (locus of enterocyte effacement) pathogenicity island (2,5,7). However, sporadic cases of HUS have been associated with over 100 different LEE-positive and LEE-negative STEC serotypes (5). In Latin America, non-O157 serotypes appear to be more commonly associated with human disease than serotype O157:H7 (12).

Outbreaks of STEC infection, with some including hundreds of cases (13–15), have been documented in at least 14 countries on 6 continents in a variety of settings, including households, day-care centers, schools, restaurants, nursing homes, social functions, prisons, and an isolated Arctic community (2,16).

HUS, the most serious complication of STEC infection, has been reported to occur with a frequency of about 8% in several outbreaks of STEC O157:H7 infection (2,16), although in one outbreak among elderly nursing home residents, it was as high as 22% (17).

The frequency of sporadic HUS in North America is about 2–3 cases per 100,000 children under 5 yr of age (2,16), in contrast to a roughly 10-fold higher incidence in this age group in Argentina (12). In South Africa (18), and in the United States (19), HUS appears to be more common in white than in black children. In England, it is more common in rural than in urban areas (10), and in Argentina, the syndrome occurs more commonly in upper-income than in lower-income groups (20,21). The reasons for these differences between population groups are not known.

3. Clinicopathological Features and Pathophysiology of STEC Infection

After an incubation period of typically, 3–5 d, the characteristic features of STEC O157:H7 infection include a short period of abdominal cramps and
nonbloody diarrhea, which may be followed, in many cases by hemorrhagic colitis, a condition distinct from inflammatory colitis that is characterized by the presence of frank hemorrhage in the stools. Fever and vomiting are not prominent features \((2,5,7)\). HUS, defined by the triad of features (acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia), develops in about one-tenth to one-quarter of the cases \((2,5,7)\). HUS may also be a complication of STEC-associated urinary tract infection \((22)\). The severity of HUS varies from an incomplete and/or a mild clinical picture to severe and fulminating disease with multiple organ involvement, including the bowel, heart, lungs, pancreas, and the central nervous system \((23)\).

The infectious dose of \(E. coli\) O157:H7 is very low (estimated to be less than 100 to a few hundred organisms). The organism is thought to colonize the large bowel with the characteristic A/E cytopathology mediated by components encoded by the LEE \((5)\). Pathological changes in the colon include hemorrhage and edema in the lamina propria, and colonic biopsy specimens may exhibit focal necrosis and leukocyte infiltration \((5,7)\). The pathogenesis of non-bloody diarrhea has yet to be fully elucidated.

Shiga toxin-producing \(E. coli\) elaborate at least four potent bacteriophage-mediated cytotoxins: Stx1 (VT 1), Stx2 (VT 2), Stx2c (VT2c), and Stx2d, which may be present alone or in combination. Stx1 is virtually identical to Shiga toxin of \(Shigella dysenteriae\), but it is serologically distinct from the Stx2 group \((7,24)\). Among the most potent biological substances known, Stxs are toxic to cells at picomolar concentrations \((24)\).

The toxins share a polypeptide subunit structure consisting of an enzymatically active A subunit (approx 32 kDa) that is linked to a pentamer of B-subunits (approx 7.5 kDa) \((24)\). After binding to the glycolipid receptor, globotriaosylceramide (Gb3) \((25)\), on the eukaryotic cell, the toxins are internalized by receptor-mediated endocytosis and target the endoplasmic reticulum via the golgi by a process termed “retrograde transport” \((24,26)\).

The A-subunit, after it is proteolytically nicked to an enzymatically active A1 fragment, cleaves the N-glycosidic bond at position A-4324 \((27)\) of the 28S rRNA of the 60S ribosomal subunit. This blocks EF 1-dependent aminoacyl tRNA binding, resulting in the inhibition of protein synthesis \((24)\).

The development of HUS is thought to be related to the translocation of Stx into the bloodstream, although the precise mechanism for this is not known \((7)\). Histologically, HUS is characterized by widespread thrombotic microangiopathy in the renal glomeruli, gastrointestinal tract, and, other organs such as the brain, pancreas, and the lungs \((7,28,29,30)\). A characteristic swelling of glomerular capillary endothelial cells accompanied by widening of the subendothelial space is seen at the ultrastructural level, suggesting that endothelial cell damage is central to the pathogenesis of HUS \((31)\). This dam-

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age is probably mediated directly by Stx after binding to a specific receptor, globotriaosylceramide (Gb3) (32), on the surface of the endothelial cell (33). The toxin is internalized by a receptor-mediated endocytic process and is thought to cause cell damage by interaction with subcellular components, which result in the inhibition of protein synthesis (24). Apoptosis may be another mechanism by which endothelial cells are damaged (34). Although the endothelial cell appears to be the main target for Stx action, there is evidence that the toxins may also mediate biological effects by interacting with other cell types such as renal tubular cells, mesangial cells, and monocytes (35–37).

The blood levels of proinflammatory cytokines, especially tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), are elevated in HUS (35–37). These cytokines have been shown, in vitro, to potentiate the action of Stx on endothelial cells by inducing expression of the receptor Gb3 (35–37).

Although the injurious action of Stxs on endothelial cells appears to be crucial to the development of HUS, the precise cellular events that result in the associated pathophysiological changes, including thrombotic microangiopathy, hemolytic anemia, and thrombocytopenia, remain to be elucidated. The contributions of various host (age, immunity, receptor type and distribution, inflammatory response, and genetic factors) and parasite determinants (infectious dose, toxin types, and accessory virulence factors) to disease susceptibility and severity remain to be fully understood (2, 5, 7). Sequencing of the genome of *E. coli* O157:H7 strain EDL 933 (in the laboratory of F. Blattner) and of its 92-kb plasmid (pO157) (38, 39), is expected to provide new insights into the pathogenesis of hemorrhagic colitis and the hemolytic uremic syndrome.

**References**


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